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Immune Related Genetic Polymorphisms and Schizophrenia Among the Chinese

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ABSTRACT: Genetic association studies were conducted among two independent cohorts of Chinese ethnicity. The samples consisted of cases and unrelated controls, ascertained from Guangzhou, China, and Singapore. The studies were prompted by our earlier report of an association between schizophrenia and HLA DQB1 alleles (HLA DQB1*0602 and HLA DQB1*0303) in the Singapore sample. Polymorphisms of HLA DQB1 and flanking markers on chromosome 6p21.3 were investigated in the first part of the study. A significant negative association with HLA DQB1*0402 was detected in the Guangzhou sample (Odds ratio, OR 0.26, 95% confidence intervals, CI 0.1, 0.6; $p < 0.02$, corrected for multiple comparisons). Additional analysis of the Guangzhou and Singapore samples revealed associations at three other anonymous markers flanking HLA DQB1. In the second part of the study, three polymorphisms at the Interleukin-1 gene cluster (IL-1, chromosome 2q13-q21) were investigated in both cohorts, since associations with schizophrenia have been reported in another sample. Persuasive evidence for an association at IL-1 was not detected in either sample. Our results suggest a susceptibility locus for schizophrenia in the HLA region among the Chinese, but further clarification is necessary. *Human Immunology 62, 714–724 (2001).* © American Society for Histocompatibility and Immunogenetics, 2001. Published by Elsevier Science Inc.

KEYWORDS: association; genetics; schizophrenia; HLA; IL-1

INTRODUCTION

Several studies have documented differences between patients with schizophrenia and suitably matched controls with respect to immunological measures such as cytokine concentrations or lymphocyte subsets [1–6]. It has also been suggested that a sub-group of patients have increased prevalence of autoimmune diseases as well as antinuclear and anticytoplasmic antibodies [1, 7, 8, 9]. The prevalence of rheumatoid arthritis and type I diabetes mellitus may be reduced among patients with schizophrenia, suggesting shared etiology between schizophrenia and these autoimmune conditions [10, 11]. Some of the results could be due to medications, but other groups have also reported increased prevalence of autoimmune diseases among medication free nonschizophrenic relatives of probands [12–16]. Thus an autoimmune pathology for schizophrenia is plausible, though persuasive evidence is unavailable [8, 16–19]. The inconsistencies may have resulted from the variable effects of other nonspecific clinical factors such as stress [20, 21]. More reliable results may be obtained if heritable immunological factors are examined.

Linkage studies form the cornerstone for identifying genes predisposing to human disease. However, genome wide linkage studies have not yielded consistent results for schizophrenia, even though the heritability is estimated at 70% [22]. The variable results may be due, among other reasons, to the presence of several genes of relatively modest effect. Candidate gene association strategies are gaining popularity for gene mapping studies because such genes may be detectable using this ap-

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proach [23]. Association studies make no assumptions about the mode of inheritance. For both reasons, they are potentially useful in schizophrenia. The present study describes studies involving immune related genes, for which associations with schizophrenia have previously been reported.

HLA and Schizophrenia

Association studies have focused on the HLA region on chromosome 6p21 for over three decades, but the results have been inconsistent [24–27]. Some of the inconsistencies may have arisen because most early studies examined HLA class I, but not class II markers. The latter are more likely to be associated with autoimmune diseases. The early studies also used serological methods, which are more error prone in comparison with PCR based assays [28].

Several recent PCR based analyses of class II markers among Caucasian and Japanese samples have reported significant associations with alleles of HLA DPB1 or HLA DRB1 [29–32]. Two recent family based studies among Caucasian samples also suggest an association at HLA DRB1 [27, 31]. Even so, several other case-control studies have failed to detect significant associations in the HLA region [26, 33, 34]. Therefore, the question of HLA association with schizophrenia is unresolved.

Over 90% of the HLA studies in schizophrenia have been conducted in Caucasian samples. It is plausible that an association is present among other ethnic groups even if it is not detectable reliably among Caucasians. Such variations have been reported for associations of HLA DQB1 alleles with type I Diabetes Mellitus [35]. Indeed, our prior studies suggest a "negative" association with HLA DQB1*0602 among African-Americans, which could not be detected among Caucasians [26, 33, 36]. In other words, the frequency of HLA DQB1*0602 was significantly reduced among African-American cases compared with control values. However, a spurious association may have occurred among the African-Americans due to known genetic admixture between Caucasian and African genes [37].

To test ethnic differences with regard to the association, an independent Chinese sample from Singapore was investigated. A significant negative association with HLA DQB1*0602 and a significant "positive" association was detected with HLA DQB1*0303 in this sample [38]. This association was also notable because the diagnostic criteria for the Chinese cases (International Classification of Diseases, 10th revision, ICD 10) were different from the African-American sample (DSM-III-R). Thus, the HLA DQB1 association appeared to be robust to diagnostic variations. Furthermore, the molecular genetic techniques employed in the studies were different, arguing against a systematic laboratory error. Taken together, these studies support an association in the HLA region among the Chinese.

Nevertheless, other considerations remain. First, the controls for the Chinese samples were unrelated adults who had been selected during a pre-employment checkup. Significant bias may be introduced by the screening process [39]. Second, the presence of hitherto unknown HLA DQB1 alleles could lead to genotyping errors. Both these possibilities were examined in the present study. DNA polymorphisms at HLA DQB1 and flanking loci were initially examined among independently ascertained cases and adult controls from Guangzhou, China. The flanking markers included short tandem repeat polymorphisms (STRPs). We reasoned that a significant association was unlikely at the STRPs due to relatively high mutation rates [40]. Nevertheless, if detected, it would provide supportive evidence for the association and potentially help narrow the region of interest. These markers were also examined in the Singapore cohort.

Interleukin-1 (IL-1) Gene Complex

Cytokines such as IL-1α, as well as the respective receptors have been localized to the brain [41]. They can modify the metabolism of neurotransmitters [42]. Because IL-1α acts as an astroglial growth factor, it may influence neurodevelopment as well as neurodegeneration [43]. Due to these diverse roles, cytokines have been suggested in the pathogenesis of schizophrenia [44]. Indeed, elevated plasma concentrations of Il-1β have been noted among drug naïve patients with recent onset of schizophrenia [5]. However, such findings may reflect epiphenomena like sleep loss or stress, rather than genuine pathogenic mechanisms [21]. Genetic evidence may be more convincing. Therefore, interest has focused on the IL-1 gene complex on chromosome 2q13-q21. This region includes three tightly linked genes: IL-1α, IL-1β, and the IL-1 receptor antagonist (IL-1 RA) [45]. Restriction fragment length polymorphisms (RFLPs) at position −889 of IL-1α, at position −511 of IL-1β and variable number of tandem repeats (VNTRs) in the intronic sequence of Il-1RA were recently investigated among Finnish cases with schizophrenia ($n = 50$) and unrelated blood donors ($n = 400$) [20]. Though significant case-control differences in allele frequencies were not detected for any of these three polymorphisms, the cases had a significant excess of individuals homozygous for the following haplotype: IL-1α allele 2/IL-1β allele 1/IL-1 RA allele 1. These findings are provocative, because the IL-1α polymorphism has functional relevance, and the IL-1 complex may be associated with ulcerative colitis as well as juvenile rheumatoid arthritis [46–48]. To test if

similar associations occur among the Chinese, both the Singapore and Guangzhou samples were examined.

MATERIALS AND METHODS

Clinical

Singapore sample. The Chinese cases and adult controls have been described earlier [38]. Briefly, the cases were male inpatients with schizophrenia (ICD10 criteria) at Woodbridge Hospital, the only psychiatric hospital in Singapore. The controls were individuals screened for a pre-employment checkup at the National University Hospital, Singapore. For all participants, ethnicity was based on self report. The sample included 171 cases and 130 controls. Informed consent was obtained from participants, in accordance with the regulations of Singapore University and the University of Pittsburgh Institutional Review Board (IRB).

Guangzhou sample. The cases were consenting inpatients with schizophrenia of Han Chinese ethnicity (DSM IV criteria). All cases were interviewed by two psychiatrists using the Structured Clinical Interview for DSM IV Axis I Disorders, Patient edition (SCID-P), [49]. The controls were unscreened unrelated adults from the same residential area as the cases. The sample included 100 cases and 99 controls. The study was approved by the Ethics Committee at Guangzhou Psychiatric Hospital and the University of Pittsburgh IRB.

Laboratory

DNA extraction. Genomic DNA was extracted from venous blood using the phenol chloroform method.

Chromosome 6p markers. In addition to HLA DQB1, five anonymous markers less than 100 kb from HLA DQB1 were analyzed. They included four short tandem repeat polymorphisms (STRPs): DQCAR2 (18 alleles), DQCAR (14 alleles), G5-1152 (15 alleles), G4-12348 (7 alleles), and G6-7571, a bi-allelic marker (Figure 1).

Genotypes at HLA DQB1 were determined using PCR amplification with sequence specific primers (SSP) [50]. The STRPs at DQCAR were typed using a PCR based assay [51, 52]. The amplified fragments were separated electrophoretically using 6% acrylamide gels and visualized using silver stain [53]. Other STRPs were analyzed using published PCR based assays [54–57]. All gel runs included as reference amplified DNA from CEPH individuals whose genotypes were known. Genotypes were read by two workers blind to the clinical status of the subject. In case of ambiguity, samples were retyped.

The marker G6-7571 was initially reported as a single strand conformational polymorphism (SSCP) [55]. To identify the sequence variation causing the SSCP, we identified four individuals with varying SSCP patterns. PCR amplified fragments from these individuals were purified using Quiagen Extraction Kits, sequenced using cycle sequencing kits (Applied Biosystems) and electrophoresed on an ABI 373 DNA Sequencer. The sequences from the variant genotypes were analyzed using multialignment sequence analysis software (CLUSTAL). We identified a T $\rightarrow$ C substitution, recognized by the restriction endonuclease MSc I. Therefore, the rest of the samples were PCR amplified, digested using MSc I, electrophoresed on 2% agarose gels and the fragments visualized with ethidium bromide.

Chromosome 2q markers. Three polymorphisms were investigated at the IL-1 gene cluster: bi-allelic markers at IL1A (-889) and IL1B (-511) and a variable number of tandem repeat marker (VNTR) at IL1RN. Published PCR based assays were employed [20, 45]. Amplified DNA fragments were visualized by electrophoresis on 2% agaorse gels followed by ethidium bromide staining.

Statistical analysis. Case-control differences in allele frequencies for bi-allelic markers were compared using Chi square tests. For comparisons involving small cells, the likelihood ratio chi square statistic was used. Such comparisons are invalid for multi-allelic markers such as STRPs, due to the large numbers of cells. Therefore, the computer program CLUMP was used [58]. Using Monte Carlo simulations, it generates tables with the same marginal totals as the table with the raw data. The number of times the chi square value from the raw data exceeds the respective values from the simulated data set is counted. This provides an estimate of the significance of the raw values. When significant case-control differences were detected thus, chi square tests were used to compare frequencies of individual alleles and odds ratios computed when significant differences were detected.

RESULTS

Analysis of Chromosome 6p Markers

HLA DQB1 (Guangzhou sample). CLUMP analysis revealed a significant association at HLA DQB1 when overall allelic distributions were compared ($p < 0.0001$, Table 1). Genotypes and allele counts for each allele were also compared among cases and controls using Chi square